HYBRIDIZATION SELF-RECOGNITION PROBE

This application is a 371 of PCT/JP00/06524 filed September 22, 2000.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to a self-recognition probe for use in detecting a nucleic acid. In particular, the present invention relates to a probe in which energy release (for example, fluorescence) from a labeling substance is controlled by an energy-absorbing substance.

Description of the Related Art

The great progress in medical genetics has been made in recent years, and decoding the entire nucleotide sequences of the human genes will soon be completed. Further, data of relationship between genetic polymorphisms and diseases have been accumulated day by day, and analysis of polymorphisms in the entire region of human genes is in progress. On the other hand, genetic tests for viruses and bacteria, which cause human diseases, have become available for practical use and contributed to medical treatment. Under these circumstances, expectation in genetic testing technology is high, and further simplification and cost reduction are needed.

Conventionally, methods for detecting a specific gene by using probes have been available. Generally, in these methods, a sample containing a nucleic acid to be detected is immobilized on a solid phase, a double-stranded chain is formed by hybridization with a nucleic acid complementary to this target (the nucleic acid complementary to the nucleic acid to be detected is referred to as a "probe" hereinafter), into which a labeling substance is introduced, and the solid phase is washed, after which the labeling substance in the nucleic acid trapped in the solid phase is detected to examine the presence or absence of the target nucleic acid in the sample. Further, in alternative detection methods, a sample nucleic acid is labeled and then allowed to form a duplex with a probe immobilized on a solid phase. These methods are well established and reliable; however, they require an excessive amount of sample, probe, reagent for detection, or the like, and the washing process is laborious,

20	EFN3-FP: 10 μl	EC1: 10 μl	20 μl	60 μl	342
21	EFN3-FP: 10 μl	EC2: 10 μl	20 μl	60 μl	985
22	EFN4-FP: 10 μl	None	20 μl	70 μl	510
23	EFN4-FP: 10 μl	EC1: 10 μl	20 μl	60 μl	508
24	EFN4-FP: 10 μl	EC2: 10 μl	20 μl	60 μl	1,042

A measuring solution (10 mM Tris-HCl, 1 mM EDTA, 50 mM NaCl, 10 ng/μl carrier DNA; 420 μl) was added to the solution treated for annealing (30 μl), and then fluorescence was measured using Shimazu RF-5000 (Ex = 494 nm, Em = 518 nm). Samples with readings exceeding the scale were appropriately diluted for measurements, and the fluorescent intensity of each original solution was calculated by appropriate conversion. The resulting fluorescent intensities are shown in Table 1~~, and also in Graph 1.~~

Some samples into which pyrene was not introduced show increased fluorescent intensity due to the formation of a double-stranded chain (No. 4 and No. 5). This is because fluorescence of fluorescein introduced into an oligonucleotide, which had been quenched by the interaction with the oligonucleotide, was released by the double-stranded chain formation. Such a phenomenon occurs often depending on sequences, and cannot be observed in No.7 through No. 12. On the other hand, in samples into which pyrene was introduced, fluorescent intensity increased only when a double-stranded chain was formed (Nos. 14, 17, 21, and 24, among Nos. 13 through 24), which shows that this probe increases fluorescent intensity by hybridization.

CLAIMS

1. A probe comprising a nucleic acid carrying a labeling substance that releases energy and an energy-absorbing substance capable of absorbing the energy released from the labeling substance, wherein energy transfer from the labeling substance to the energy-absorbing substance is intercepted by the hybridization of the probe with a target nucleic acid.

2. The probe according to claim 1, wherein the energy is photo energy.

(Amended)

3. The probe according to claim 1 ~~or 2,~~ wherein the labeling substance is selected from the group consisting of a fluorescent substance, a delayed fluorescent substance, and a chemiluminescent substance.

(Amended)

4. The probe according to ~~any one of~~ claims 1 ~~to 3,~~ wherein the energy-absorbing substance is an intercalator or a substance which specifically binds to a double-stranded nucleic acid.

5. The probe according to claim 4, wherein the intercalator is selected from the group consisting of acridine, anthracene, pyrene, and derivatives thereof.

(Amended)

6. The probe according to claim 1 ~~or 2,~~ wherein the labeling substance is fluorescein, and the energy-absorbing substance is selected from the group consisting of pyrene, coumarin, and acridine.

(Amended)

7. A solid phase carrier for detecting a nucleic acid, on which the probe of ~~any one of~~ claims 1 ~~to 6~~ is immobilized.

(Amended)

8. A method for detecting a nucleic acid comprising the steps of contacting the probe of ~~any one of~~ claims 1 ~~to 6~~ with a nucleic acid sample and then measuring energy released from the labeling substance.